Vol. V. *August, 1903.* *No. 3*

BIOLOGICAL BULLETIN.

ON THE CONDITIONS GOVERNING THE PRODUCTION OF ARTIFICIAL PARTHENOGENESIS IN ARBACIA.

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In a previous paper[1] it was shown that sea-water concentrated by evaporation to a definite volume would produce parthenogenesis in eggs of the sea-urchin, *Arbacia*, subjected to its influence for a given length of time. During the continuation of these experiments for the purpose, primarily, of observing the morphological phenomena there has become evident a series of conditions necessary to a high ratio of parthenogenetic development. These conditions are briefly — purity of solutions, stage of development of ovarian eggs when placed in the concentrated sea-water, length of time the eggs are to remain in this solution, temperature of solutions. This article is based upon observations on eighty-three experiments, fourteen of them performed between July 25 and August 14, 1901, and sixty-nine between July 4 and August 15, 1902. The work of both seasons was pursued at the Marine Biological Laboratory, Woods Holl. In every instance the eggs for each experiment were taken from one female only. In this brief preliminary account detailed references to individual experiments are in some cases given, not to illustrate the condition peculiar to that experiment alone, but rather to set forth prevailing conditions.

Solutions.— These are rendered ineffective, (1) by the presence of foreign substances; (2) spermatozoa; (3) excessive number of eggs. The eggs of *Arbacia* are extremely sensitive to foreign substances liable to be introduced by the use of glassware not thoroughly cleansed or glassware previously used as receptacles

[1] Hunter, *American Journal of Physiology*, VI., 1901, p. 177.

for chemicals. For this reason it was found advisable to use only new glassware.

Much time was consumed in the work of sterilization to prevent contamination from spermatozoa. This, however, is essential. In the eighty-three experiments referred to sterilization was performed in accordance with the plan mentioned in the paper cited. If, after this treatment, there were eggs that developed through normal fertilization, such escaped notice. In five other experiments made to determine the necessity of this sterilization, the sea-water was not sterilized nor was the sea-urchin carefully washed in hydrant water. In these five experiments a few normally developing forms were noted.[1]

The relative proportion of eggs to concentrated sea-water is an important factor in determining the percentage of development. In the paper referred to mention was made of the necessity of placing comparatively few eggs in the solution. Tabulated results of some experiments on this point make the relative value of this condition more apparent. In two bowls containing equal amounts of condensed sea-water there were placed in the first bowl a greater number of eggs and in the second bowl very few. Out of the first bowl 14 per cent. reached the swimming gastrula stage; out of the second bowl 87.5 per cent. reached the same stage. Notes taken on early stages of the culture showed that in the bowl containing the less number, segmentation began in a greater number of eggs.

In other experiments an endeavor was made to measure approximately the number of eggs placed in a given amount of sea-water. One of these experiments is placed in tabulated form.

No. 1, 1 pipette full of eggs in 50 c.c. concentrated sea-water.

No. 2, 5 pipettes full of eggs in 50 c.c. concentrated sea-water.

No. 3, 10 pipettes full of eggs in 100 c.c. concentrated sea-water.

After two hours, transferred to sterilized sea-water, frequently changed at first — examined twenty-seven hours later with results as follows:

[1] The test of purity of culture was based on the facts, (1) that cleavage in parthenogenetically developing eggs of *Arbacia* at no time prior to blastula stage, resemble normal processes; (2) the shortest time in which any culture under constant observation reached the active swimming stage was nine hours and seven minutes, under like conditions normally fertilized embryos become active in about six hours; (3) the absence of the perivitelline membrane.

No. 1, 7 gastrulæ out of 16, 43 per cent.

No. 2, 9 gastrulæ out of 90, 10 per cent.

No. 3, 10 gastrulæ out of 109, 10 per cent.

Examined again twenty-six hours later with the following results :

No. 1, 8 plutei and 12 gastrulæ out of 27, 74 per cent.

No. 2, 1 pluteus out of 42, .02 per cent.

No. 3, no living forms out of 39.

This difference in the ratio of development is probably due to the noxious effects of the undeveloping eggs in the solution. This being the case a frequent change of the concentrated solution might raise the percentage, for while the sterilized sea-water was changed repeatedly the concentrated water was not changed at all during the period. The difference in results was evident at this stage, that is, the two lots of eggs when removed from the condensed solution showed a difference in behavior. As just noted, the eggs from the cultures containing the smaller number showed a larger percentage of segmentation. Cultures No. 2 and No. 3, having same ratios, gave similar percentages.

State of Development. — Wilson [1] has observed that the eggs of *Toxopneustes* which would not fertilize with spermatozoa gave some of the best results obtained with the magnesium solution. Delage [2] notes in *Strongylocentrotus* that frequently eggs which will not develop by artificial parthenogenesis are readily fertilized by spermatozoa. A number of observers have noted the wide variation in the behavior of the eggs of different females. Some eggs do not develop at all, others give large percentages of active larvæ. A case in point : An experiment with a large female, the eggs of which by their number, color and the freedom with which they came from the ovary — the ovaries in such cases when placed in sea-water lose form and become a mass of eggs — seemed to signify that the eggs were fully matured (oötids). As further proof, the greater part of these eggs were fertilized with spermatozoa, this resulted in the normal development of all the eggs observed. The remainder of the eggs were subjected to the influence

[1] E. B. Wilson, " A Cytological Study of Artificial Parthenogenesis in Sea-urchin eggs," *Archiv f. Entwickelungsmech.*, XII., 4, 1901, p. 535.

[2] Delage, Y., " Études Expérimentales sur la Maturation Cytoplasmique Chez les Echinodermes," *Archiv d. Zoöl. Exper. et Generale*, 3, Ser., IX., 1901, p. 300.

of condensed sea-water for two hours and four minutes, resulting in the appearance of many cytasters (Wilson) and the segmentation of many eggs, but no active larvæ. Segmentation and development ceased after four hours in the sterilized sea-water. The ovaries of another female were teased in sea-water and a small number of pale eggs were obtained. From the condition of the ovaries, the color and the number of eggs it was evident that the eggs were not mature (oöcytes). These were placed for the same length of time in concentrated sea-water and then transferred to normal sea-water. More than 90 per cent. of these eggs reached the active larval state. Eggs of females brought directly from the bed of the ocean gave better results than those kept in the laboratory aquarium for a time. The higher temperature of the sea-water in the laboratory probably hastened maturation. It became evident throughout the later series of experiments that oöcytes gave satisfactory results and oötids gave negative results. It seems probable, therefore, that concentrated sea-water is effective in producing development in *Arbacia* only when its influence is brought to bear upon the oöcyte.

The interesting question naturally arises concerning the exact stage at which the solution is effective. If this influence causes retention of the second polar body and its assumption of the rôle of the spermatozoön the subject is at once brought into direct relation with Boveri's[1] theory of natural parthenogenesis. In Delage's[2] observations on the influence of carbon dioxide on *Asterias* he gives results to show that the moment of susceptibility of the eggs lies between the time when the nuclear membrane of the germinal vesicle begins to dissolve and the beginning of the resting period of the egg nucleus; and that the immediate cause does not concern the polar bodies but rather the suspension of maturation for a given period. Upon resumption the polar karyokinesis is not confined to one region of the egg, but instead becomes general and includes the whole egg. Consideration of this phase is curtailed by Delage's[3] own statement

[1] Th. Boveri, "Zellstudien," I., 1887, p. 73.

[2] Y. Delage, "Nouvelles Recherches sur la Parthenogenese Experimentale chez Asterias glacialis," *Archiv de Zoöl. Exper.*, 1902, p. 217.

[3] Y. Delage, "Etudes Experimentales sur la Maturation cytoplasmique chez les Echinodermes," *Archiv de Zoöl. Exper.*, 3 Ser., IX., 1901, p. 295.

that phenomena manifested in the starfish must not be assumed to occur in the sea-urchin, and further according to the same author[1] the eggs of *Strongylocentrotus* are mature before being subjected to the solution. From this it would seem that there is a difference in the behavior of the eggs of *Arbacia* and *Strongylocentrotus*. In *Arbacia* it did not appear that in the development of the egg there was only one opportune moment when the concentrated solution was effective, but rather that ovarian eggs placed in the concentrated solution, were influenced to maturate and that maturation brought about in this way resulted, when the eggs were removed to normal sea-water, in segmentation and subsequent development. Experiments with eggs apparently mature frequently give small percentages, one to five per cent. of larval development. This may be accounted for by the presence of a few oöcytes in the ovaries. The difficulty in the case of *Arbacia* is that owing to the opacity of the eggs it is not possible to ascertain their exact state when placed in the concentrated solution.

There is some evidence which probably bears upon the question to be found in the examination of sections. Without entering into a detailed description at this time we find in iron-hæmatoxylin sections a heavily staining body in contact with the nuclear membrane. In some cases astral rays extend out from this dark body. In others these rays are absent. Later prophases occur, such as the elongation of the nucleus with an aster at each pole, followed by the mitotic figure in its various phases. In other words, there appear to be processes closely resembling normal karyokinesis. This conspicuous dark body shows its attitude towards the nucleus in cases where the dark body has failed to divide. In such cases, the nucleus is elongated on the side of contact and the chromatin is aggregated on the same side. It seems reasonable, therefore, to say that in these parthenogenetic eggs there is a force whose behavior approximates that of the spermatozoön.

Briefly, then, in the sea-urchin egg maturation takes place in the ovary before normal oviposition.[2]

[1] *Ibid.*, pp. 296, 301, 324.
[2] E. B. Wilson, "The Cell," 1900, p. 236.

The eggs used in these experiments were not deposited naturally, but were from ovaries removed from the female.

The ovaries thus taken were of two kinds: first, dark red in color, delicate in structure; when placed in sterilized sea-water eggs flowed freely from them without cutting or teasing; second, light red in color, firm in structure; comparatively few eggs obtained even after ovaries are cut and teased.

The eggs from ovaries of the first class gave unsatisfactory results when subjected to influence of concentrated sea-water, satisfactory results when fertilized with spermatozoa.

The eggs from ovaries of the second category have given percentages as high as 80 to 90, of parthenogenetic swimming forms, when subjected to influence of concentrated sea-water for the proper period.

Sections through ovaries, typical of this second class, reveal large numbers of oöcytes determined as such by the presence of the prominent germinal vesicle. Sections through thirty-two different follicles were examined. Only those showing germinal vesicle (oöcytes) or egg-nucleus (oötids) were counted. In these thirty-two sections of follicles there were 183 oöcytes and 85 oötids.[1] Oöcytes much smaller than normal eggs were not counted. The percentage of forms developed parthenogenetically is thus shown to bear a direct relation to the number of oöcytes in the culture.

It seems reasonable, then, to infer that the concentrated sea-water acts effectively upon the oöcyte only. The exact nature of this action it is hoped subsequent study will determine.

Duration.—The eggs of *Arbacia*, as is well known, are not sufficiently transparent to permit close observation upon the activity of the cell contents. For that reason I have been unable to note in the egg any definite appearance which would signify the proper moment for transference from condensed sea-water to sterilized sea-water. In a few cases I have found wide variations in the time that the eggs can be transferred and yet develop. The shortest time was one hour and twenty-two minutes. The limits within which the eggs from a given culture could be removed and yet develop were

[1] These follicles were from the ovaries of one female. Of the utilized eggs from this female fully 75 per cent. became swimming larvæ.

relatively narrow. It seems that the critical moment does not lie, as in the case of Delage's observations on *Asterias*, in the time when the eggs are placed in the solution, but rather when they are removed from the solution.

The stage of development of eggs when placed in solution evidently has some bearing on the time required for development. The differences in the states of ovarian eggs would seem to account for the differences in the time required for development, not only for the eggs of different individuals, but as in the experiment given below, for the eggs of the same individual.

The culture just referred to, the one in which larval development was obtained after an hour and twenty-two minutes in the concentrated solution, was one of a series of experiments to determine the proper length of time and also the time in which eggs of a given female will develop. This experiment also presented the longest period of time within which eggs of the same individual could be removed from the concentrated solution for subsequent development. The eggs were placed in the concentrated sea-water and allowed to remain one hour. Watch-glass cultures of approximately equal number of eggs, the standard being three pipette drops of eggs in each watch glass, were removed every two minutes from the concentrated solution and placed in sterilized sea-water. The length of time that eggs remained in the concentrated solution is given and opposite are the observations made, beginning seven hours and twenty minutes later.

Minutes in Concentrated Solution.	Notes Taken Seven to Ten Hours Afterward.
62.	No segmentation.
64.	A number of fragments from a few eggs that had segmented and then broken.
66.	The same.
68.	The same.
70.	Fragments more abundant but nothing in the nature of a cluster of blastomeres.
72.	The same.
74.	The same.
76.	Not so many fragments, a few eggs segmented into two and three blastomeres.

78. Very few whole eggs, nearly all in fragments of halves and less sizes.

80. Blastomeres remaining together, few fragments.

82. Three active well-formed blastulæ (examination made ten hours after removal from concentrated sea-water).

82–98. The eight cultures taken out during this time showed about the same percentage of development as 82.

100. No segmentation in this culture nor in any of the subsequent cultures.

This experiment shows a duration of sixteen minutes within which eggs were removed and larval development ensued. This was the widest range of the series. In many experiments a difference of five minutes on either side of the optimum moment determined the life of the culture. In all cases, as noted by other observers, eggs removed from the concentrated solution after a brief period begin to segment but do not continue to develop until they reach the swimming blastula stage. Eggs permitted to remain too long plasmolyze when placed in sterilized sea-water. As a result of this series of experiments the optimum period was determined at two hours. In each case three cultures were formed of the eggs, one of five minutes before the period, one at the period, and the other five minutes after the two hours.

Temperature. — The most favorable temperature obviously is the normal temperature of sea-water. Sudden changes caused by the use of water of a different temperature for replenishing cultures is detrimental. Greeley[1] has shown that blastulæ can be developed parthenogenetically in concentrated sea-water at a temperature of 2°, 11° and at the room temperature of 23°. I am convinced that uniform results cannot be obtained from cultures kept on the laboratory table. The changes in temperature which occur between day and night materially affect the behavior of the eggs. For this reason towards the close of the season the bowls containing the solutions were surrounded by running sea-water. This insures constancy of temperature as well as approximates the normal temperature.

[1] A. W. Greeley, Biol. Bulletin, IV., No. 3, p. 132.

SUMMARY.

1. The conditions governing the production of artificial parthenogenesis in *Arbacia* by the use of sea-water concentrated by evaporation to a definite volume, are purity of solutions, stage in development of ovarian eggs, duration in concentrated solution, temperature of solutions.

2. The efficacy of solutions is subject to the presence of foreign substances, spermatozoa, relative number of eggs in a given amount of concentrated sea-water, and temperature. Foreign substances are excluded through extreme care in the preparation of solutions; spermatozoa are eliminated by raising normal sea-water to 70 degrees, by sterilizing all instruments in the flame, by washing thoroughly the body of the sea-urchin and the hands of the operator for three brief periods under stream from the hydrant. Results are most constant at normal temperature of sea-water. Development is obtained at room temperatures 22° to 24°. Variations in temperature of solutions materially affect the development of the culture.

3. The concentrated solution appears to be effective in producing development in oöcytes only. By reason of the opacity of the egg it is difficult to ascertain the exact stage or subsequent behavior in concentrated solution.

4. The average optimum period for eggs in concentrated solution lies between one hour and fifty-five minutes and two hours and five minutes.

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April 4, 1903.

HETEROGENY AND VARIATION IN SOME OF THE COPEPODA OF LONG ISLAND.

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In the spring of 1898, my attention was attracted to certain of the Copepoda that occur in large numbers in the fresh-water ponds in some of the outlying districts of Brooklyn. The material, which contained many *Cyclops*, was collected soon after the ice had disappeared from the surface of these shallow pools and even at this early season most of the *Cyclops* were large and carried eggs in all stages of development.

I isolated individuals with eggs, and subsequently observed numerous color-changes, which accompanied the rapid growth and extrusion of eggs into the egg-sacs. A single instance will suffice to show the rapidity of these changes, and the fertility of the individuals. On the 19th of April, 1898, a *Cyclops*, carrying dark blue eggs, was isolated. On the 20th dark bluish ova could be seen through the transparent body-wall, making the body appear dark, while the dark eggs in the egg-sacs had developed into embryos of a reddish tint. On the 22d the copepod carried dark eggs again, and the body was again almost colorless, with a faint streak on either side, still marking the position of the ovaries. On the 23d it remained unchanged. On the 24th the body was again dark but no eggs were attached. On the 25th the dark eggs were carried in appended sacs and the body was again colorless. On the 26th the dark eggs became detached. On the 27th the body again appeared dark. There is no record in my notes for the next two days, but when I again looked at the copepod the body was colorless. While it carried no egg-sacs, the ova must have been discharged since the last record on the 27th instant.

I attempted to identify the form, which agreed with *C. parcus* (Herrick), in most of the points that are regarded as species-characteristics but it differed from *C. parcus* in the number of its antennal segments.

The chief morphological features by which species of *Cyclops* are recognized are the following :

1. The number of joints in the antennæ.
2. The number of joints in the rami of the four swimming feet.
3. The armature of the swimming feet.
4. The number of joints in the fifth foot, which is rudimentary.
5. The shape and armature of the segments of the fifth foot.
6. The structure of the abdomen with the caudal stylets and the armature of the caudal stylets.
7. The shape of the receptaculum seminis.
8. The armature of the maxillipeds.
9. The relation between the length of the antennæ and the cephalothorax.

The characteristics of *C. parcus* are as follows :

1. Seventeen-jointed antennæ.
2. Three-jointed rami in the swimming feet.
3. Armature of the last segment of the swimming feet.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
2 outer spines.	1 outer seta.	2 outer spines.	1 outer seta.
2 apical setæ.	1 apical spine.	1 apical spine.	1 apical spine.
	1 apical seta.	1 apical seta.	1 apical seta.
2 inner setæ.	3 inner setæ.	3 inner setæ.	3 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
Like second.		2 outer spines.	1 outer seta.
		1 apical spine.	
		1 apical seta.	2 apical spines (equal).
		3 inner setæ.	2 inner spines.

4. Two-jointed fifth foot.
5. The basal joint short and broad with a single seta on the outer margin.

A long, cylindrical, distal segment with a blunt, inner spine and a long, outer seta, but very slightly plumose.

6. The abdomen is composed of segments, the first of which is as long as the remaining segments combined. The caudal stylets are long.
7. The receptaculum seminis is broadly oval.
8. There are four hairs on the distal segment of the larger branch of the maxillipeds. The second segment has a large, immovable dactyl with a row of teeth along the edge, and with a

small hair at its base. Attached to the immovable dactyl is a small, movable one.

9. The antennæ are about the length of the cephalothorax.

The points in which the Long Island *Cyclops* that I have studied differs from *C. parcus* are: In the number of antennal segments, there being 13 instead of 17, and in the occasional variation in the armature of the outer ramus of the fourth foot, there being but one outer spine and one seta, where *C. parcus* has typically two spines; as well as in the armature of the terminal joint of the large ramus of the maxilliped, where two small hairs replace one large one; also in the armature of the distal joint of the fifth foot, which carries an outer hair, in place of the unserrated spine which is present in the form with seventeen joints in the antennæ.

As the correlated characteristics of species occur with great regularity in the *Cyclops*, and as the form under consideration seemed, both on account of its relatively large size and its fertility, to be a mature form, I searched for similar individuals but for a long time failed to find them.

In the summer of 1899, I had the opportunity of collecting large numbers of *Cyclops* at Cold Spring Harbor, L. I., where several fresh-water ponds afford excellent opportunities for the study of a variety of species. Though I have worked over some of this material with great care, I have never met with a single instance of a thirteen-jointed antenna.

In March of the present year, 1903, I again met with a number of *Cyclops* having thirteen-jointed antennæ. This material was collected in one of the large, shallow, fresh-water ponds at Jamaica, Long Island. The copepods were found in great numbers hidden beneath the fallen leaves along the edges of the pond. Again I noticed marked color-changes incident to the development and laying of ova. Some were red in the body and carried blue eggs in their paired sacs, while many were dark in color and carried about the partly developed reddish embryos.

Associated with these larger forms were smaller *Cyclops*, often without eggs, and emerald green to the naked eye, owing to the numbers of green protozoa that had attached themselves to the cuticle and almost concealed the host. The larger *Cyclops* with

the pink bodies and the blue eggs, or *vice versa*, were comparatively free from the one-celled forms. I believe this fact is important as pointing to the strong probability of a recent moult. Further study revealed the fact that the larger forms had invariably seventeen segments in the antennæ and that they agreed in all essential details with the species known as *C. parcus* (Herrick).

After formulating data gathered from the study of species-characters in many different individuals from the same locality, I was able to clearly distinguish three groups, in all of which, all the leading species-characteristics of *C. parcus* (Herrick) were combined with a varying number of segments in the antennæ, which, however, all belonged to the same type (Fig. 1).

Group I. comprised individuals with thirteen antennal segments.

Group II. comprised individuals with fourteen antennal segments.

Group III. comprised individuals with seventeen antennal ments.

Nearly all of the *Cyclops* referred to as covered by protozoa and hence appearing green, belong to Group II. or are intermediate between Groups I. and II., and are characterized by antennæ with fourteen segments either fully formed, or in process of forming. I have studied no less than ten individuals which show clearly that the fourteen-jointed antenna is derived from the thirteen-jointed one, by the division of the tenth segment — the fourth from the distal end of the antenna — which is divided almost equally into halves by a transverse partition.

It is *always* the tenth segment which is dividing at this stage, and in all cases recorded, when the two antennæ are not in the same stage of division, it is without exception the *left* that is in advance of the right, in which division can still be seen in progress, as in Fig. 1, *B*.

I know of no explanation of the retarded division in the right antenna, and it may be a mere coincidence that all of my observations agree on this point. One *Cyclops* in which the fourteen segments were perfectly formed in both antennæ, proved particularly interesting, for I believe it furnishes positive proof

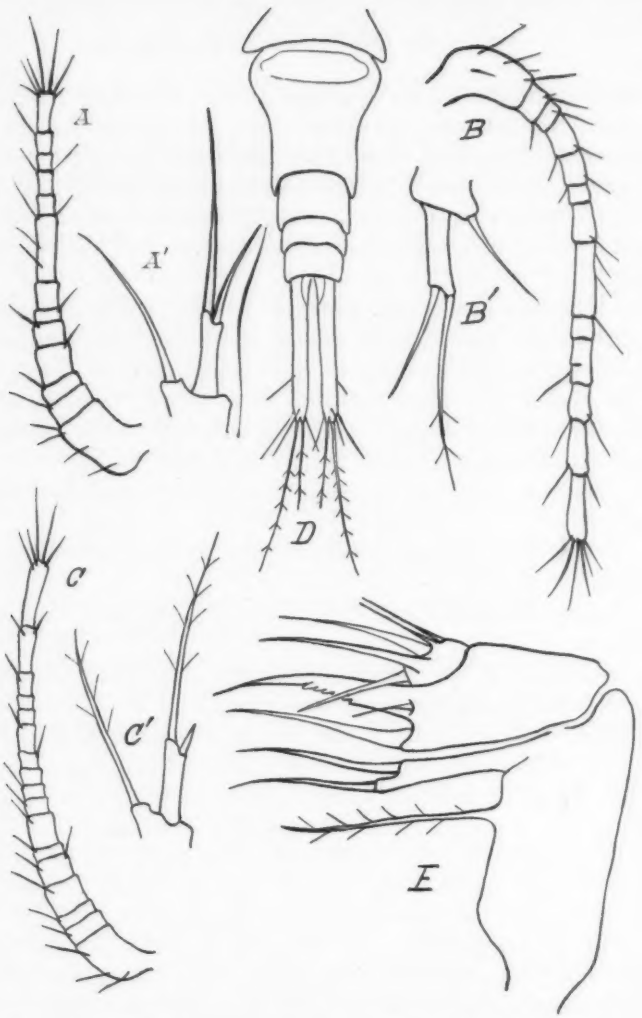

FIG. 1. Shows the antenna and fifth foot of a cyclops with thirteen-jointed antennæ, *A*, *A'*, Group I. The antenna and fifth foot of a cyclops with fourteen jointed antennæ, *B*, *B'*, Group II. The antenna and fifth foot of a cyclops with seventeen-jointed antennæ, *C*, *C'*, Group III. *D* shows the abdomen, the receptaculum seminis and the caudal stylets characteristic of all the forms with thirteen, fourteen or seventeen-jointed antennæ. *E* shows the large ramus of the maxilliped characteristic of the three forms. *B* also shows the tenth antennal segment in the act of dividing, thus giving rise to the fourteen-jointed antenna.

As compared with the length of the cephalothorax all the antennæ *A*, *B* and *C* shown in Fig. 1 are relatively long, extending to the first segment irrespective of the number of segments they contain.

that this apparently stable individual with the fourteen-jointed antennæ represents but a temporary condition in the development of a form with seventeen antennal segments. In the case referred to, the long eighth joint, that is characterized by three rather widely separated setæ, showed distinct, transverse lines across the segment at the level of each of the two lateral setæ. Half way up the remaining section, a slight indentation in the cuticle marked the position of the wall that completes the separation of this long segment into four small ones of almost equal size.

The breaking up of the eighth segment in the manner indicated by these markings gives to the seventeen-jointed antenna a short eighth segment with a single distal seta; a short ninth segment with a distal seta; a short tenth segment without any armature, and a short eleventh segment with one distal seta. These are precisely the conditions which prevail in the seventeen-jointed antennæ.

In his report on "The Entomostraca of Minnesota," Herrick describes a *Cyclops* strikingly like the one from Jamaica, Long Island, *with fourteen-jointed antennæ, three-jointed rami with the armature of the last joints like that given for C. parcus, and with a two-jointed fifth foot* "with the armature like *C. strenuus*, which also resembles *C. pulchellus*." The stylets are very long. These correlated peculiarities of structure are recognized as constituting a distinct species known as *Cyclops insignis* (Claus). Herrick mentions that "in a previous edition it was suggested that this is but an atavistic form of *C. pulchellus* — *C. strenuus*." If *C. strenuus* is to be regarded as practically the same form as *C. abyssorum*, as Schmeil suggests, the Long Island form can hardly be brought into relation with it, for the armature of the swimming feet, which is remarkably constant in forms of equal size, differs markedly in the two cases. Schmeil, however, seems to attach little importance to this fact.

That the Long Island form with the fourteen-jointed antennæ represents a transitional stage in the development of a seventeen-jointed form, there can be little doubt, though the determining of the species in the terms of an old and confused classification is by no means an easy matter. The length of the caudal stylets

is relatively greater in the *Cyclops* with the fourteen-jointed antenna than in the adult *C. parcus*, though in *C. parcus* the stylets are characteristically long. Another slight difference is seen in the presence of a hair in the form with the fourteen-jointed antennæ, in place of a small spine on the inner angle of the distal joint of the fifth foot of the seventeen-jointed form. Compare *A'*, *B'*, *C'*, Fig. 1. Moreover, the distal joint of the so-called *C. insignis* is strikingly long, longer than the corresponding joint in *C. parcus*. The difference between the two forms seems to be almost entirely one of proportion and size, the *insignis*-like form being slightly smaller than *C. parcus*, and often with fewer or no eggs.

In favor of the existence of a separate species for those forms with fourteen-jointed antennæ, and against the suggestion made by Herrick that *C. insignis* represents a transitional stage in development, Schmeil urged the occurrence of the *Cyclops* in large numbers, and its relatively large size, both of which *observations* I can confirm. I can not, however, agree with Schmeil's interpretation; although the form is abundant and moderately large, it is often, though not always, without eggs either in the body or attached, when older forms associated with it are remarkably prolific. Moreover, if studied at the right stage, the form with fourteen segments in the antennæ gives frequent signs of being still in a period of growth characterized by morphological changes. The fact that the smaller form is densely covered by foreign growths indicates that it has not very recently moulted. In this connection it may not be irrelevant to allude to a few observations made on isolated copepods.

I separated a number of *Cyclops* in a small watch crystal. All were about the same size, some green to the naked eye, some dark, and others carrying eggs. A few days later my attention was drawn to a bright red *Cyclops* with a perfectly clean cuticle. It had seventeen segments in the antennæ, and from the absence of protozoa on its surface it must have moulted quite recently. I then looked about in the dish for cast-off skins and found one still well covered with protozoa and having fourteen-jointed antennæ.[1]

[1] Inasmuch as there were other individuals in the watch crystal, this is by no means *conclusive* proof that the seventeen-jointed form had shed the fourteen-jointed skin, but I could find no other explanation of its presence in the dish and I offer the fact for whatever it is worth.

I then set aside six *Cyclops* with fourteen-jointed antennæ, giving them clean hydrant water containing but little food and some fresh-water plants. At the time of their separation two had fourteen segments only in the left antenna, while the right antenna of each contained a dividing segment, the tenth from the base of the antenna, or the fourth from the distal end. Two weeks later the division of the segment was still incomplete, showing that in this case at least, the formation of partition walls is not very rapid. The bodies looked lighter and clearer than before, and I examined them again to see if any changes had taken place, but none had occurred.

In his explanatory notes accompanying Plate XXXIV.[1] which shows the species-characteristics of *C. parcus* (Herrick), Herrick shows "caudal stylets of an elongate form," in Fig. 3, with which my own drawings agree perfectly. It is quite possible that the elongated distal segment of the fifth foot may be a mere variation correlated with the elongation of the caudal stylets in Herrick's 'elongated form' of *C. parcus* which he suggests "is to be regarded as a post-imago."

A single characteristic which Herrick describes for *C. pulchellus*, but of which no mention is made in the characterization of *C. parcus*, to my knowledge, is the presence of serrations on the distal margins (ventral and lateral) of the last abdominal segment, while the remaining margins of the abdominal segments are free from such markings. All of the individuals of the three groups — *i. e.*, of the thirteen-, the fourteen- and the seventeen-jointed antennæ — agree with *C. pulchellus* in having these serrations, while Groups I. and II. also agree in having "two rather long setæ" which are not at all or only slightly plumose on the terminal segment of the fifth foot. But they all differ from *C. pulchellus* in not having the basal joint of the fifth foot longer than wide; the basal joint is unequivocally wider than it is long, and in this respect agrees with *C. parcus*.

Although the armature of the appendages is very constant in the Cyclopidæ, it is quite common to meet with similarly placed spines and setæ of different lengths. A notable instance of this

[1] "Copepoda, Cladocera and Ostracoda of Minnesota," Zoölogical Series, II., 1895, of the Geological and Natural History Survey of Minnesota.

occurs in the armature of the third joint of the large ramus of the maxillipeds of the fourteen-jointed and seventeen-jointed forms. The armature usually consists of three large hairs and two very small ones growing close together at the base of one of the large hairs (Fig 1, *E*). In the fourteen-jointed forms, these two small hairs are strikingly shorter than they are in the seventeen-jointed form. With this single exception, the maxillipeds are precisely alike in both groups.

I am aware that Herrick describes the armature of the terminal segment of the larger branch of the maxilliped of *C. parcus*, as consisting of four hairs. I have found an instance in which four large hairs of almost uniform size occur, but a more frequent condition in the Long Island *Cyclops* is seen in those instances which show three large hairs and two short ones, in place of the four hairs of Herrick (Fig. 1, *E*).

Among the many *Cyclops* I have studied, I have seen but one with eighteen segments in the antennæ. In this case the eighteenth segment is derived from the seventh segment, by transverse division, at the level of the seta. In both right and left antennæ the division is incomplete, extending but half way across the segment.

I have studied this *Cyclops* with great care, and in every detail of structure, it agrees perfectly with the forms associated with it in showing the chief species-characteristics of *C. parcus*.

I have repeatedly made written records of body-segments and appendages showing the complete armatures, and have made many outline drawings of those parts that are correlated in the determination of species, and I believe no room for doubt remains that the *Cyclops* with thirteen and with fourteen antennal segments, as well as the form with eighteen segments, are all to be referred to the type with seventeen segments in the antennæ. Those having thirteen and fourteen segments, known as *C. insignis*, though very abundant forms and though sexually mature, do not represent a group of sufficient permanency to warrant us in regarding them as representatives of a distinct species. They are rather to be considered as transitory stages which, though capable of producing young, have not as yet attained their maximum growth, or their highest degree of complexity.

The *Cyclops* with the eighteen-jointed antennæ agrees with Claus' description of *Cyclops elongatus*, so far as Herrick has quoted Claus. Nevertheless, its close agreement in all species-characteristics with *C. parcus*, with which it was found, and the very exceptional occurrence of so many antennal segments, make it highly probable that we are dealing here with a case of variation rather than with a species-character.

The *Cyclops* from Cold Spring Harbor, Long Island, were collected at the surface of a very shallow pond along a road-side near the laboratory of the Brooklyn Institute. The pond was choked with water-plants and a scum of duck-weed floated on the surface. From the extreme shallowness of the pond, any life there must have been exposed to rapidly changing conditions. The material collected in this pond was all taken from one locality within a radius of a few feet, where the copepods were in among the duck-weed.

I attempted some statistical studies in variation on these forms, but the work was soon interrupted by the comparatively small number of individuals belonging to the same species, or to species closely enough related to warrant any use of them in obtaining data. Most of the forms I have been wholly unable to identify, for while they agree with well known species in certain characteristics, they differ from them in others which are apparently no less important.

Certain combinations of characters occur so frequently, that, in the absence of transitional forms, one is often tempted to believe that in the bewildering array of forms before him, he is dealing with new variations, of which it is almost impossible to say whether they have a species value or not. Whether the forms met with illustrate pædogenesis, or whether the season was connected in any way with the morphological aspect of the copepods, I cannot say, not having been able to collect from this vicinity at any other season. But I have not seen any transitional stages in an individual such as would warrant the linking of it with any well known species.

One *Cyclops* frequently met with, combines the following characteristics: *Antennæ nine-jointed; rami of swimming feet two-jointed; rudimentary fifth foot one-jointed.*

ARMATURE OF THE SWIMMING FEET.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	1 apical spine.
1 apical seta.	1 apical seta.	1 apical seta.	1 apical seta.
4 inner setæ.	5 inner setæ.	4 inner setæ.	5 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	2 apical spines.
1 apical seta.	1 apical seta.	1 apical seta.	
4 inner setæ.	4 inner setæ.	4 inner setæ.	3 inner setæ.

The antenna and fifth foot of this form are seen in Fig. 2. The pravalence of the form alone is not sufficient reason for

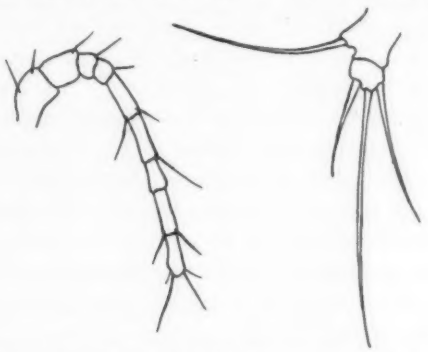

FIG. 2. Shows the antenna and the fifth foot of a cyclops with nine antennal segments. The fifth foot is two-jointed and resembles the fifth foot of the cyclops with the ten-jointed antennæ.

regarding it as a distinct species, and the probability is that we are here dealing with a transitional stage in the development of a species with a greater number of antennal segments, as seen in the case of the fourteen-jointed form, for no species in its mature condition is recognized as having nine antennal segments, while the fact that *the rami are two-jointed and the number of setæ on the last joint of the inner ramus is exceptionally large*, suggests that the rami may subsequently acquire a third joint. Moreover, the armature of the feet is strikingly like the armature of another *Cyclops* having ten antennal segments.

This second form which occurs frequently in the same locality,

combines the following characteristics: *Antennæ ten-jointed; rami of swimming feet two-jointed; rudimentary fifth foot two-jointed.*

ARMATURE OF THE SWIMMING FEET.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
2 apical spines.	1 apical spine.	1 apical spine.	1 apical spine.
3 inner setæ.	1 apical seta.	1 apical seta.	1 apical seta.
	5 inner setæ.	4 inner setæ.	5 inner setæ.
Third Foot.		**Fourth Foot.**	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	1 apical spine.
1 apical seta.	1 apical seta.	1 apical seta.	1 apical seta.
4 inner setæ.	4 inner setæ.	4 inner setæ.	3 inner setæ.

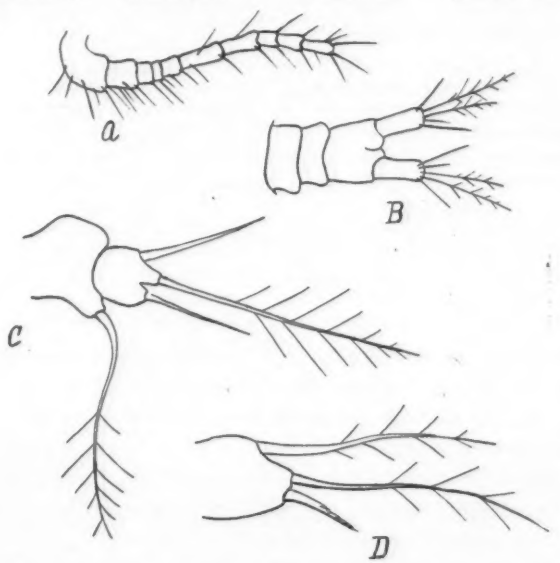

FIG. 3. The antennæ, the abdomen and caudal stylets and the two types of fifth foot correlated with the 10-jointed antennæ. *C* shows the fifth foot correlated also with the 9-jointed antennæ, while *D* shows the fifth foot correlated with the 11-jointed antennæ. *B* represents the type of abdomen and stylets correlated with 10 antennal segments irrespective of the form of the fifth foot.

Wherever these forms with the nine- and ten-jointed antennæ occur they show the same striking similarity in the armature of the swimming feet. *The nine-jointed forms are perfectly constant*

throughout the group, but *the ten-jointed forms vary considerably within the group*, occasionally combining three-jointed rami with a two-jointed fifth foot, and occasionally two-jointed rami with a one-jointed fifth foot.

According to Herrick's classification of the Cyclopidæ, there is but one species having ten-jointed antennæ, *i. e.*, *C. phaleratus*, which may combine either *ten- or eleven-, usually eleven-jointed, antennae with three-jointed rami in the swimming feet, and with a one-jointed fifth foot.* I have found this combination in a single case, and the antennæ contained each eleven segments. Herrick gives only the formula for the fourth foot of *C. phaleratus*, with which the above form also agrees. The entire armature of the terminal joints of the four swimming feet in the Cold Spring Harbor form is shown below.

First Foot.		Second Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	3 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	1 apical spine.
1 apical seta.	1 apical seta.	1 apical seta.	1 apical seta.
3 inner setæ.	3 inner setæ.	4 inner setæ.	3 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	2 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	2 apical spines.
1 apical seta.	1 apical spine.	1 apical seta.	
4 inner setæ.	3 inner setæ.	4 inner setæ.	2 inner setæ.

The length of the antenna in *C. phaleratus* as compared with the cephalothorax is short, whereas in the Cold Spring Harbor form the antennæ are relatively long, extending nearly to the second thoracic segment. Moreover, in a single instance the long second joint of the antenna showed a light, transverse band near its proximal margin, suggesting the characteristically short second segment of the eleven-jointed antenna.

The chief characteristics of the cyclops with the eleven-jointed antennæ are *three-jointed rami in the swimming feet combined with a two-jointed fifth foot* (Fig. 4).

Herrick recognizes three species having eleven antennal segments; one of these is a European form of marked peculiarity; a second is *C. diaphranus*, whose species-characteristics are *eleven-jointed antennæ, two-jointed rami in the swimming feet, and a one-jointed fifth foot, with a long seta and one short spine.*

I have not found a single *Cyclops* combining these characters. The eleven-jointed antennæ are, with one exception, so far as my studies show, always correlated with *three*-jointed rami in the

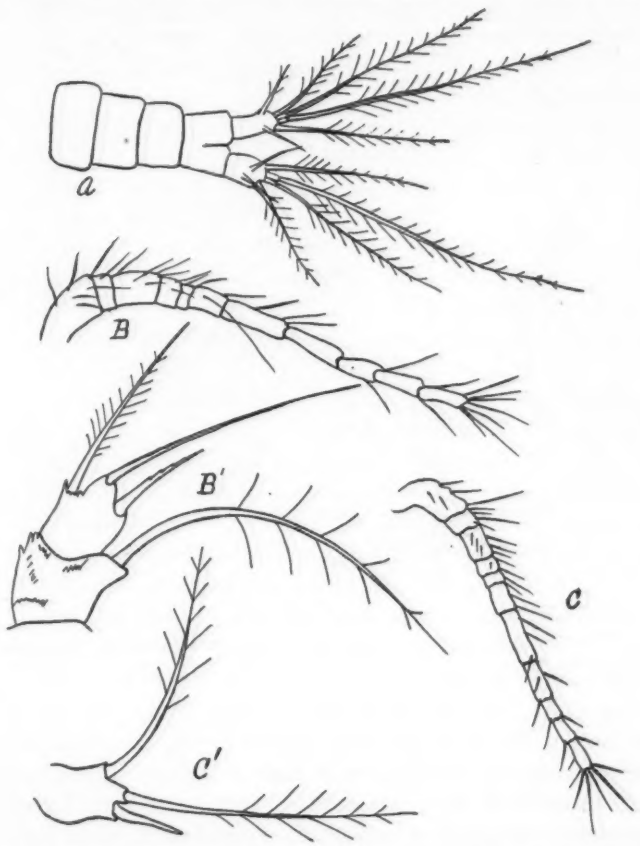

FIG. 4. Represents an eleven-jointed antenna, *B*, correlated with a two-jointed fifth foot *B'* and short caudal stylets with very long, plumose setæ, *A*. *C* and *C'* represent an eleven-jointed antenna and a correlated one-jointed fifth foot with the same abdomen and stylets as are seen in the form with the eleven-jointed antennæ and the two-jointed fifth foot.

swimming feet, and the armature of these forms is precisely like that of *C. phaleratus*, whether the fifth foot be one-jointed or two-jointed. The third species having eleven-jointed antennæ which

Herrick recognizes, also combines a one-jointed fifth foot with two-jointed rami. It is known as *C. affinis* and is like *C. phaleratus*, "which it closely resembles."

A fourth and a last type to which I shall refer, is seen in a not infrequently occurring form *which combines twelve-jointed antennæ with three-jointed rami in the swimming feet and a two-jointed fifth foot* (Fig. 5).

Herrick recognizes three species as having these characteristics, namely: *C. capillatus* and *C. crassicaudis*, both European

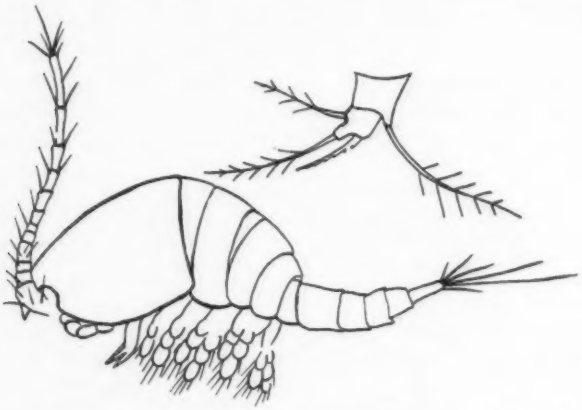

FIG. 5. Shows a twelve-jointed antenna which is relatively very long as compared with the cephalothorax, notwithstanding the relatively small number of antennal segments present.

forms, and *C. varicans*, an American form. The two former are described as Scandinavian forms only. Of the third species *C. varicans*, Herrick says that it is "the American species most nearly resembling the European form with twelve antennal segments and a two-jointed fifth foot." "Unhappily," Herrick also remarks, "this species was taken but once." On Plate XXX.[1] Herrick figures the first foot of *C. varicans*, which he pictures as having two-jointed rami in the swimming feet. Herrick explains that the last joint is homologous to two fused segments, and that the separation might take place "at the next moult." The form I have studied shows the armature when the rami have reached

[1] "Copepoda, Cladocera and Ostracoda of Minnesota."

the three-jointed condition, and the reduction in the number of spines and setæ in the armature of the fourth foot might seem to bear out Herrick's suggestion.

C. Varicans. Fourth Foot.		Cold Spring Harbor Cyclops. Fourth Foot.	
Outer Ramus.	*Inner Ramus.*	*Outer Ramus.*	*Inner Ramus.*
3 outer spines.	1 outer seta.	2 outer spines.	1 outer seta.
1 apical spine.	1 apical spine.	1 apical spine.	2 apical spines.
1 apical seta.	1 apical seta.	1 apical seta.	
4 inner setæ.	4 inner setæ.	4 inner setæ.	2 inner setæ.

The armature of the Long Island form suggests *C. phaleratus*, though in the first foot it is not identical.

I have found three of these forms among a relatively small number of individuals and they agree very closely with one another, the only difference being in a slight variation in the armature of the swimming feet, a spine occasionally appearing in place of a seta.

Supposing that these individuals represent *C. varicans*, the Cold Spring Harbor form is very evidently in a later stage of development than the individual figured by Herrick. Any appeal to relative ages as an explanation of differences, requires the supposition that some of the segments of the feet have an adult armature while other segments have not. But there is no reason for supposing that the number of spines and setæ in the fourth foot is incident to the breaking up of the rami into three segments instead of two, for the armature of the first foot is not reduced by the presence of the additional joint in the rami.

Summary.

The Long Island *Cyclops* (*C. insignis?*), having fourteen-jointed antennæ, three-jointed rami in the swimming feet, with two-jointed fifth feet and elongate caudal stylets, is a transitional stage in the development of a seventeen-jointed form *C. parcus* (Herrick?). The eighteen-jointed antenna is derived from the seventeen-jointed form by division of the seventh segment.

Out of fifteen individuals taken at random, none of whose antennal segments exceed twelve, five precisely similar individuals constitute a group having *nine antennal segments, two-jointed rami* and *two-jointed fifth feet.*

Four individuals constitute a second group having typically *ten antennal segments*, *two-jointed rami*, and *two-jointed fifth feet*. Two of these individuals show marked variation, one in having *three-jointed rami* in the swimming feet, the other in having a *one-jointed fifth foot*.

Four individuals constitute a third group, characterized by *eleven-jointed antennæ*, *three-jointed rami*, and *two-jointed fifth feet*. One member of this group has a *one-jointed fifth foot*, and *this is the only individual out of the thirteen that can be given any place among species*, *i. e.*, *C. phaleratus*, as combining well recognized species-characters.

Three individuals constituting a fourth group combine the following characteristics: *twelve-jointed antennæ*, *three-jointed rami*, and *two-jointed fifth feet*. These forms suggest *C. varicans*, with which they have much in common, but from which they differ considerably in detail.

Some facts point to the probability that the Cold Spring Harbor forms with the ten-jointed antennæ are morphologically undeveloped. Especially does the variation within the group consisting of but few individuals point to the instability of these forms.

What the true nature of these correlated peculiarities in *Cyclops* may be, can only be determined by following the life history of each individual. The relatively large size of these forms, and the frequency with which they occur, as well as the constancy of the correlated characteristics, suggest on first acquaintance with the Cyclopidæ, that they represent distinct species, but a fuller acquaintance warns us to look further for an explanation of these most perplexing variations which are doubtless largely due to the acquiring of sexual maturity while the morphological changes in the body are still incomplete, and to the varying external conditions to which they are subjected.

BROOKLYN, NEW YORK,
March 30, 1903.

ON THE OCCURRENCE AMONG ECHINODERMS OF LARVÆ WITH CILIA ARRANGED IN TRANSVERSE RINGS, WITH A SUGGESTION AS TO THEIR SIGNIFICANCE.

CASWELL GRAVE.

In this paper a short account is given of some observations made at the laboratories of the United States Fish Commission at Woods Hole and Beaufort on the larvæ of various echinoderms. The attempt is also made to show that these observations, taken together with those made by other students of the group, have a direct bearing upon one phase of the problem of the early ancestry of the echinoderms.

It would be quite impossible to give an intelligible discussion of the bearing these observations are interpreted to have upon this subject without first recalling the hypotheses which have been put forward by other students of the group to account for its origin and present organization.

The hypothesis which now seems to have the most general acceptance is not the work of any one mind but represents the work of many. It would be difficult, therefore, in giving a hasty review of its most important points, to credit each of its authors with just his contribution, so I shall make only such comments in passing as will serve to explain the changes and additions which seem to me to be warranted.

Observations.

Holothurians.

The barrel-shaped pupæ of Holothurians have been long known, having been described by Müller,[1] Semon[2] and others. They arise in each case by the breaking up and rearrangement of

[1] J. Müller, "Abhandlungen über die Larven und Metamorphose der Echinodermen," *Abl. Kgl. Akad. Wiss. Berlin.*

[2] R. Semon, "Die Entwicklung der Synapta digitata, und die Stammesgeschichte der Echinodermen," *Jena Zeitschr.*, Vol. XXII., 1888.

the ciliated bands of the fully formed auricularian larvæ at the time when the metamorphosis into the adult form is about to take place. Semon's figures of the auricularian larva and the pupa

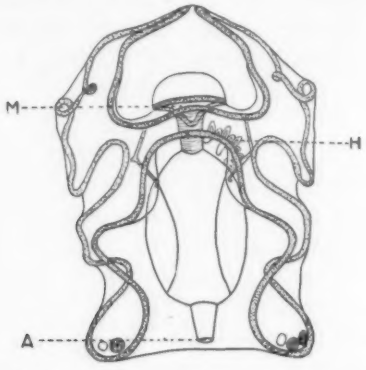

FIG. 1. Auricularian larva of *Synapta digitata*. After Semon. *M*, mouth; *H*, hydrocœle; *A*, anus. The ciliated bands stippled.

of *Synapta digitata* are reproduced in outline in Figs. 1 and 2. During the pupal stage the mouth shifts from a ventral to a terminal position and the tentacles and tube feet first become func-

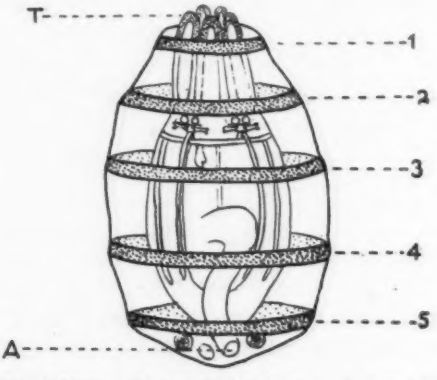

FIG. 2. Pupa of *Synapta digitata*. After Semon. *A*, anus; *T*, tentacles; 1, 2, 3, 4 and 5, ciliated rings.

tional. The ciliated rings of the pupa are five in number and are arranged transversely to its long axis.

Selenka[1] has studied and figured the larva of *Cucumaria doliolum* which, although totally unlike an auricularian larva, can be well compared with a pupa. It is an elongated free swimming creature with four, sometimes five, transversely arranged ciliated rings, in addition to which, at the anterior end, there is a ciliated field. This ciliated field is one of the first of the larval structures to disappear as development progresses. In Selenka's figure of this larva, reproduced in outline in Fig. 3, five tentacles and two

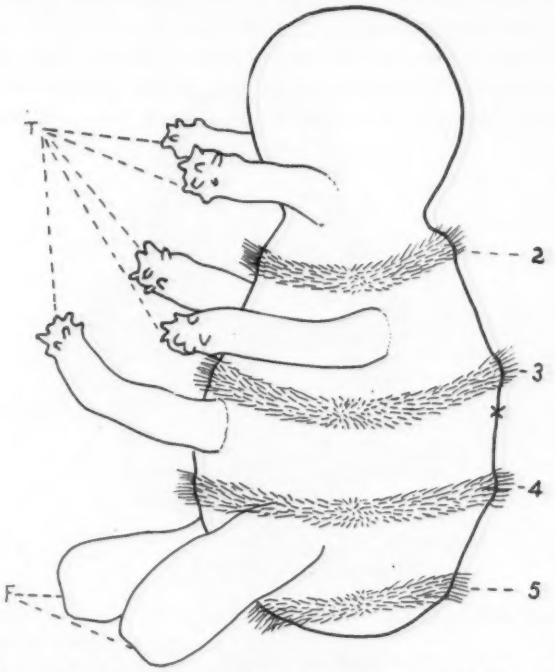

FIG. 3. Larva of *Cucumaria doliolum*. After Selenka. *F*, tube feet; *T*, tentacles; 2, 3, 4 and 5, ciliated rings.

tube feet are shown to be developed and the rotation of the mouth and tentacles to the terminal position has begun. The eggs of *C. doliolum* are quite large and well supplied with yolk,

[1] E. Selenka, "Zur Entwicklung der Holothurien," *Zeit. f. wiss. Zoöl.*, Vol. XXVII., 1876.

thus differing widely from the small transparent eggs of *Synapta digitata*. The efficient locomotor and feeding apparatuses with which the larva of the latter species is provided, enabling it to care for itself, are not needed by the larva of *Cucumaria doliolum* for whose care provision has already been made. The larva of *Cucumaria* can, as it were, give its whole attention to the production of a creature with the structure of the adult while the larva of *Synapta* must make this secondary to food getting.

Crinoids.

In *Antedon rosacea*, the only species of crinoid the development of which has been studied, the eggs are supplied with considerable yolk and for a time the developing larvæ are brooded.

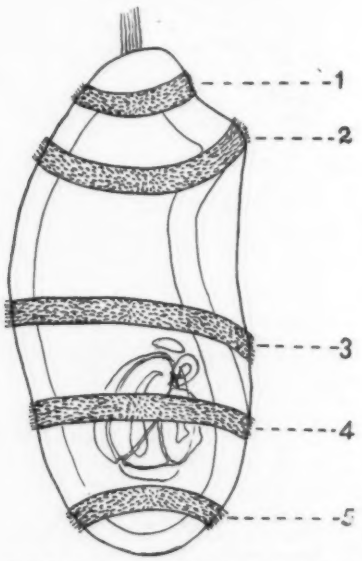

FIG. 4. Larva of *Antedon rosacea*. After Seeliger. Internal organs shown in posterior end. 1, 2, 3, 4 and 5, ciliated rings.

The free-swimming period is of short duration and the development is more or less direct. The larva is elongated and cylindrical and is encircled by five transverse ciliated rings. An

apical tuft of longer cilia is also present. Seeliger's[1] figure of it is reproduced in outline in Fig. 4. No pore canal is developed at this stage but the point on the hydrocœle at which it will appear later, I have indicated by a small *x*.

Ophiurids.

For a long time the larvæ mentioned above were the only observed cases in which the ciliated bands are arranged in transverse rings, and they were considered to have no special significance. Since 1899, however, I have found three other cases

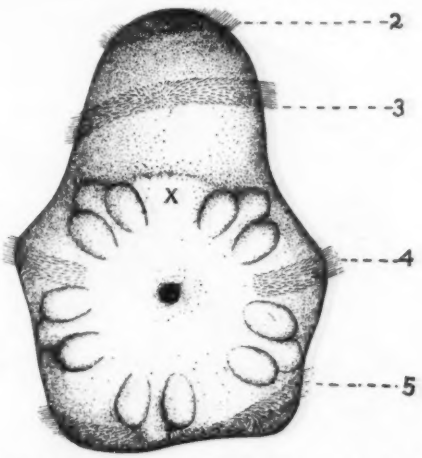

FIG. 5. Ventral view of the young larva of *Ophiura brevispina*. Original. 2, 3, 4 and 5, ciliated rings.

which exhibit the same peculiarity and which represent two other classes of echinoderms.

The larva of *Ophiura brevispina*, which I[2] described in 1899, is well supplied with yolk and very early in its development it sinks to the bottom and clings to grass blades where it undergoes its late larval stages and final metamorphosis. It is a larva without arms or processes of any kind and no skeletal rods such

[1] O. Seeliger, "Studien zur Entwicklungsgeschichte der Crinoiden," *Zoöl. Jahrb.*, Bd. VI., 1892.

[2] Caswell Grave, "Ophiura brevispina," *Mem. Natl. Acad. Sci.*, 1900.

as are found in ophiuran plutei are developed, although at one time I mistook the beginnings of the skeletal plates of the adult for such. The anterior end of the larva is produced into a long preoral lobe about which two ciliated rings are developed. The posterior end is enlarged and contains the various internal structures of the larva and developing ophiurid. The mouth is ventral and interrupts the third ciliated ring of the larva (numbered 4 in Fig. 5). The fourth ring (5) surrounds the posterior end. The dorsal pore is situated at the point indicated by the small x between ciliated rings 3 and 4. As development progresses the preoral lobe diminishes in size until finally it is entirely ab-

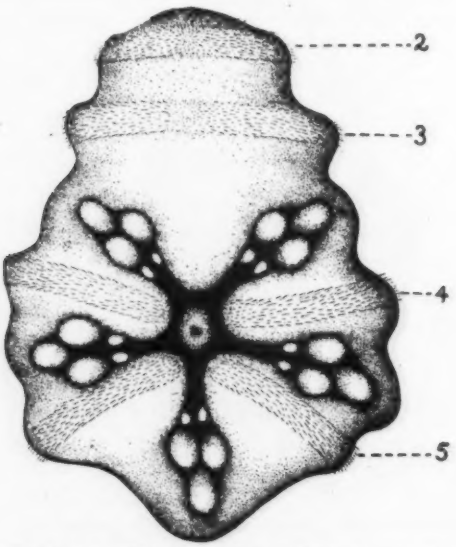

FIG. 6. Older larva of *Ophiura brevispina*. Original. The change in position which takes place in the ciliated ring (5) is shown.

sorbed. During late larval life a change in the arrangement of one of the ciliated rings also takes place. The ring numbered 5 becomes interrupted on the ventral side and takes on a more definite relation to the mouth (see Fig. 6).

In 1900 I found a second ophiuran larva at Beaufort which, in its metamorphosis from the pluteus to the radial form, showed the

same tendency to rearrange the ciliated bands into transverse ciliated rings which is found among the holothurians. The outline of the pluteus is shown in Fig. 7. When the developing ophiurid has become quite large and the tissues of the pluteus are being absorbed, the ciliated bands of certain of the arms become applied to the disc in a quite definite manner, viz., about the madreporic interradius which had an anterior position in the larva, a complete ring is formed; an interrupted ring is laid down between rays 5 and 4 on one side and 1 and 2 on the other. A third ring crosses the base of ray 3. Not until I had examined a

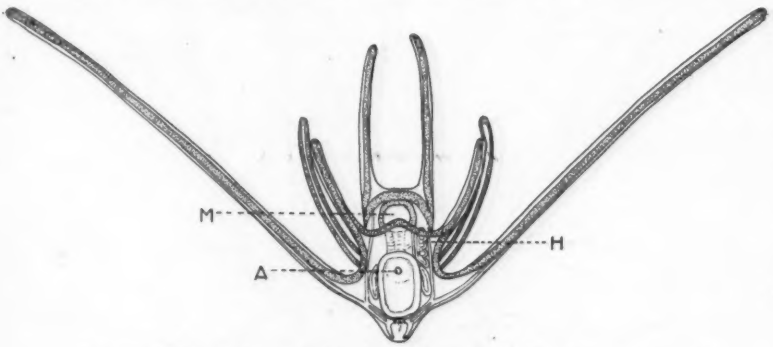

FIG. 7. Ophiuran pluteus (sp.?) from the "tow" at Beaufort. *A*, anus; *M*, mouth. *H*, hydrocœle. Ciliated bands stippled.

number of these metamorphosing plutei was I satisfied that this arrangement of the ciliated areas was not accidental but in all cases examined (a dozen or more) the arrangement was practically the same as that shown in Fig. 8.

Echinoids.

During the summers of 1900, 1901 and 1902 I succeeded in rearing large broods of the larvæ of *Mellita testudinata* from the fertilized egg to the form in which the adult structure is attained. The larva is a typical highly specialized pluteus as will be seen from the outline of Fig. 9. The just metamorphosed *Mellitas* all showed three parallel transverse ciliated rings; the middle one of which is interrupted by the mouth (see Fig. 10). The function of these ciliated rings in the young *Mellitas* is probably to

assist them in feeding until the tube feet have grown sufficiently to assume the function.

The Hypothetical Bilateral Ancestor.

Although numerous papers have been written on the subject of the phylogeny of the echinoderms there are but few which retain their vitality at the present time. In these, notwithstand-

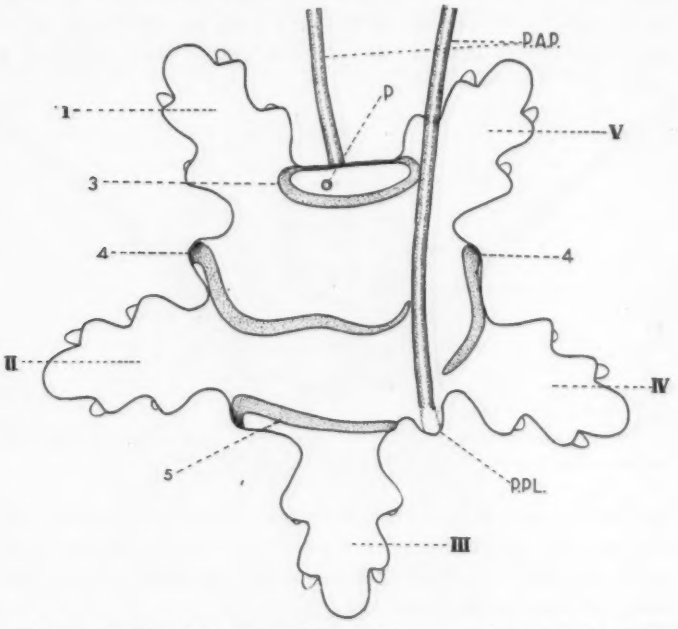

Fig. 8. Outline of the young ophiuran which metamorphoses from the pluteus shown in Fig. 7. Original. I., II., III., IV. and V., arms of the ophiuran; 3, 4 and 5, ciliated rings. *P. Pl.*, remnant of the posterior end of the pluteus. *P. A. P.*, long posterior arms of the pluteus which are never absorbed but are finally dropped. *P.*, madreporite.

ing the fact that many differences in detail exist, there is a very great similarity in the views set forth and I may state in this connection that the facts of this paper and many of my unpublished observations are an additional support to the hypothesis which has been gradually developed by Bury, McBride and

Bather, and serve to carry it one step further. Each of these students has reconstructed the hypothetical ancestor both in its bilateral free swimming stage and the stage during which it became radially symmetrical. The same plan is followed in this paper.

The papers of Bury,[1] McBride[2] and Bather[3] in which the hypothetical bilateral ancestor of the echinoderms is recon-

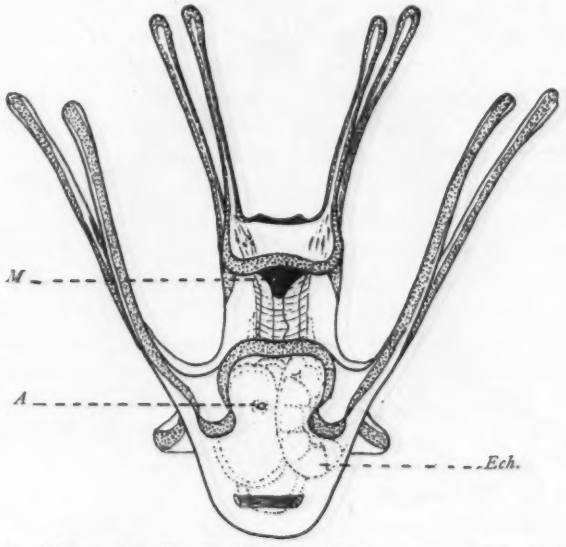

FIG. 9. Pluteus of *Mellita testudinata*. Original. *A*, anus; *Ech.*, developing sand dollar; *M*, mouth. Ciliated bands stippled.

structed and figured, are so well known and the reasons for every detail of the anatomy of the creature are therein so well set forth that it would be a waste of the reader's time to again do more than give an outline of the supposed structure of the hypothetical organism, discussing such points only in which a change is made.

[1] Henry Bury, "The Metamorphosis of Echinoderms," Q. J. Mic. Sc., No. 149, 1895.

[2] E. W. McBride, "The Development of Asterina Gibbosa," Q. J. Mic. Sc., No. 151, 1896.

[3] F. A. Bather, "A Treatise on Zoology." Part III., "The Echinoderma." Edited by E. Ray Lankester, 1900.

Briefly then, the earliest ancestor of the group of echinoderms of which there is much trustworthy evidence, was a free-swimming organism of microscopic size with an elongated body and a long preoral lobe. At the tip of the preoral lobe a sense organ and

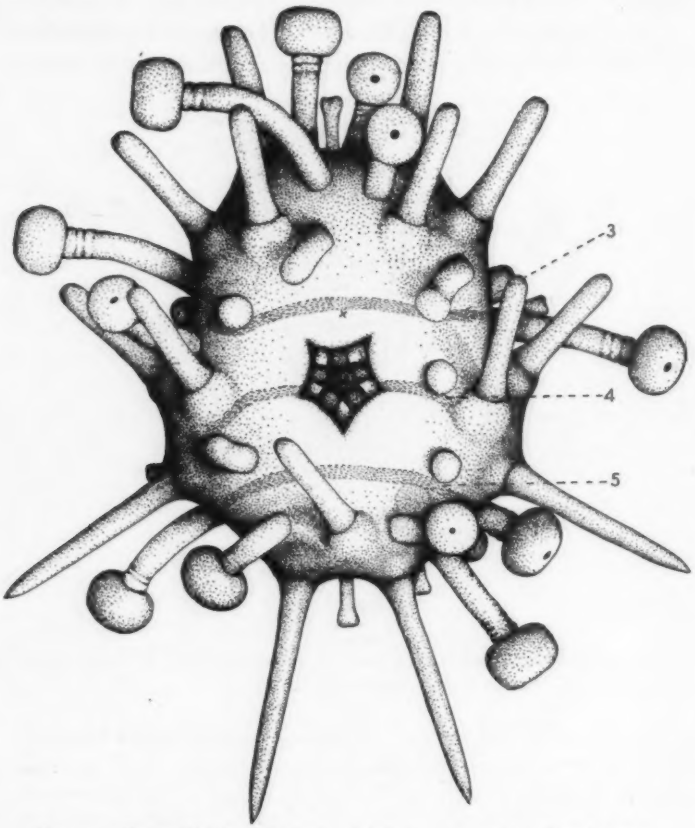

FIG. 10. Young *Mellita testudinata*. Original. 3, 4, and 5, ciliated rings.

nerve center was located. The contours of the body were plain, no arms or processes of any kind being present. The alimentary tract occupied the posterior part of the body, the mouth and anus opening ventrally. Three pairs of body cavities, arranged symmetrically with reference to the alimentary tract, were present.

The anterior pair extended into the preoral lobe where it may have been united into a single cavity. Posteriorly its cavities were placed on the right and left of the œsophagus and each cavity opened to the exterior, on the dorsal surface of the animal, through a ciliated duct. The posterior end of each anterior cavity was connected with the corresponding cavity of the middle pair by a second duct, also ciliated. The middle cavities were situated on either side of the point of union of the œsophagus and stomach. The posterior cavities were larger than those of the anterior and middle pairs and were applied to the stomach, forming a mesentery on the dorsal mid line.

If Fig. 11 *a*, of this paper is compared with Bury's Fig. 45, McBride's Fig. 157 and Bather's Fig. 1 it will be seen that the general plan is the same with differences in detail only.

Bury's idea that the hydrocœle (left middle body cavity) encircled the œsophagus (the right cavity having entirely disappeared) even during the period of the free-swimming existence of the animal, is, in the light of recent observations, an unnecessary assumption and one for which no explanation has been made. The changes which take place in the *posterior* pair of body cavities of echinoderm larvæ, by which the *left* one becomes horseshoe-shaped and encircles the stomach, are almost exactly similar to those by which the *left middle* body cavity takes on the form of a ring surrounding the œsophagus. If to explain the former it is necessary, as Bury and others believe, to assume a shifting of the position of the mouth and œsophagus incident to a life on the bottom, then a similar explanation for the latter is also called for. I agree with the more recent writers in the assumption that both the hydrocele and left posterior body cavity acquired their circular shape and position around the alimentary canal, at the same time, viz., during the period when the entire organization of the animal was being readjusted to its new conditions of life on the bottom.

According to McBride's hypothesis, each of the middle body cavities possessed, during the free swimming stage of the ancestor, five tentacles which were used in the capture of food. There is good evidence for the existence of two hydrocœles (middle body cavities), as McBride has shown in his work on

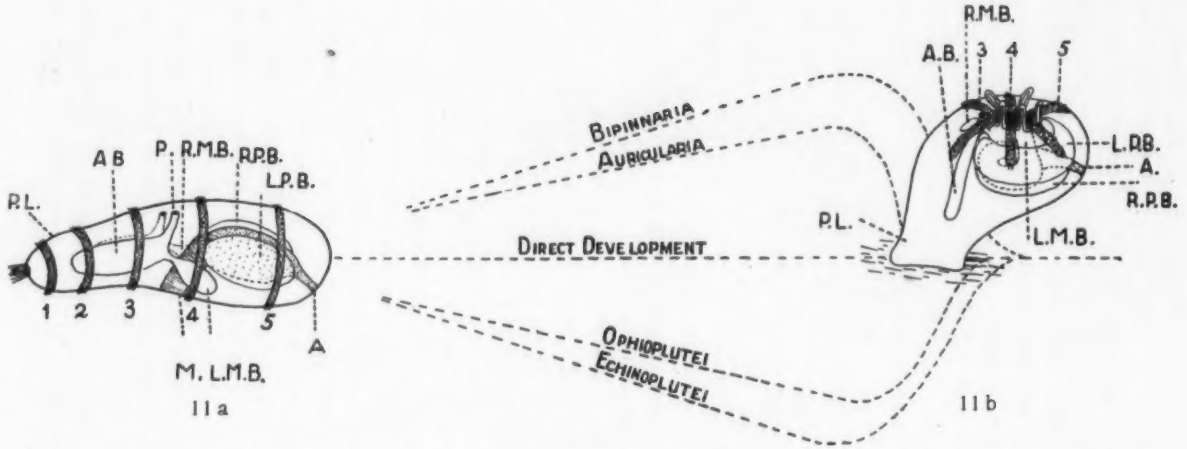

FIG. 11. Scheme to illustrate the suggestions of this paper concerning the struture and appearance of the hypothetical free-swimming and fixed ancestors of the echinoderms and the relation which the type of development of the more familiar echinoderm larvæ bears to the type which is represented by the larvæ of *Antedon*, *Cucumaria* and *Ophiura*. 11*a*, the hypothetical free-swimming ancestor. 11*b*, the hypothetical fixed ancestor. *A*, anus; *A.B.*, anterior body cavity; *L.M.B.*, left middle body cavity (hydrocœle); *L.P.B.*, left posterior body cavity; *M*, mouth; *P.*, dorsal pore; *P.L.*, preoral lobe; *R.M.B.*, right middle body cavity; *R.P.B.*, right posterior body cavity; 1, 2, 3, 4 and 5, ciliated rings.

Asterina, and as is further demonstrated in the pluteus of *Mellita*, but that they possessed tentacles or assisted in capturing food *at this time* is not, I think, supported by evidence. The structure and function of the left hydrocœle as an organ of locomotion and feeding was, in my opinion, acquired during the period of sedentary life after the animal had so increased in size that ciliary action alone was not equal to the work of food gathering. This point will be more fully discussed, however, further on.

To the bilateral ancestor as above described I would add, in the place of the general coat of cilia with which it is usually provided, a locomotor and feeding apparatus consisting of five transversely-placed ciliated rings and an apical tuft of sensory cilia. The position of each is shown in Fig. 11, *a*.

Semon[1] in his discussion of the larva of *Synapta digitata* concludes that the transverse ciliated rings of both the holothurian pupæ and the larva of *Antedon* are to be considered as secondary structures. Lang[2] also states that it is not at all likely that the ciliated rings (of echinoderm larvæ) have any phylogenetic significance. From the facts then available this was the only conclusion warranted and its has been accepted almost without exception by zoölogists. It seems to me however that the observations recorded in this paper, which have been made since the publication of the works of the authors just mentioned, make it worth while to again call attention to the question and to discuss the bearing which the accumulated facts have upon our conception of the structure and history of the hypothetical pelagic ancestor of the echinoderms.

Larvæ such as *Auricularia*, *Bipinnaria*, *Brachiolaria* and *Plutei* have never, to my knowledge, been seriously consided to be primitive although the attempt to establish a relationship between echinoderms and *Balanoglossus*, on account of the general similarity of the movements and external characters of the *Auricularia* and *Tornaria* larvæ, comes very near to an implied belief in their primitiveness. In each of the above-mentioned larvæ we

[1] Richard Semon, "Die Entwicklung der Synapta digitata und die Stammesgeschichte der Echinodermen." *Jena Zeitschrift*, Bd. XXII., 1888.

[2] Arnold Lang, "Text Book of Comparative Anatomy," p. 546. (Eng. trans.)

have to do with highly specialized organisms, there being a long period in the life of each during which it is thrown upon its own resources. Its existence during this period depends upon its ability to procure food and escape from its enemies. The rigorous selection which must take place under these conditions can not have failed to have had a profound effect upon the whole organization of the larvæ and especially upon their external characters.

The whole tendency has been however to look for primitive characters among free-swimming larvæ, throwing aside those which are brooded or otherwise cared for as much more likely to be modified and secondary. If my suggestion as to the significance of the larvæ of echinoderms with transverse rings is correct then this view is incorrect. On the other hand we would expect to find the least modified development among larvæ which are freed, to a greater or less extent, from the task of caring for themselves, provided in such cases the eggs have not been so crowded with nutritive materials as to become greatly enlarged or that, during the brooding, no connections with the mother are established or protective structures developed. Kenogenetic characters are no doubt found in both types of larvæ and the problem is to ascertain which has remained truer to the ancestral form.

The final, sudden and complicated metamorphosis into the adult form which is so characteristic of *free-swimming* larvæ is good evidence that they have been carried far out of the path of phylogeny. In larvæ without a long independent existence the metamorphosis is gradual and such as might be expected if it is in any way a true picture of the past history of the race.

No very great similarity is shown in the external forms of the familiar types of echinoderm larvæ and it is difficult to think of any one of them as having been the type from which the others originated, but it is possible to think of them all as having arisen from a type of larva such as is found in *Antedon*, *Cucumaria* and *Ophiura*.

Owing to the similarity in position of the ciliated rings with reference to the other organs of the body of the larvæ of the above-named species, and all other cases in which ciliated rings

have been found, a very definite homology can be shown to exist between them, as I have endeavored to indicate by the numbers which have been placed opposite the rings in each of the drawings. It is conceivable that the long arms of *Auricularia*, *Bipinnaria*, and the various *plutei* may have arisen and been developed from elevations of the ectoderm beneath certain parts of the ciliated rings, the result of which would have been an increase in their length and thereby an increase in their efficiency in locomotion and feeding. The relation which the type of directly developing larvæ of *Antedon*, *Cucumaria* and *Ophiura* is suggested to bear to the familiar types, is shown in Fig. 11 ; the larvæ with transverse ciliated rings being considered the primitive condition from which the other larvæ have been specialized and carried far out of the path of phylogeny, as a result of their independent life. To this type of development the specialized larvæ tend to return at the time when their free-swimming life is given up for one on the bottom, as is indicated in holothurian pupæ, a certain ophiuran larva and young *Mellitas* in all of which the transverse ciliated rings reappear at the time of metamorphosis in the form in which they were of functional importance to the common ancestor during the early period of its life on the bottom.

All larvæ have not deviated to the same extent from the direct line, as is shown not only in their less complicated structures but also in the less radical readjustment of organs which takes place during their metamorphosis. In ophiurid plutei, for example, the larval mouth and œsphagus are taken over as such into the adult form, which, as has been pointed out, must have been the case in phylogeny. In echinoid plutei, however, the specialization which has taken place in these organs has been carried so far that it is impossible to readapt them to the needs of the adult and new ones must be formed.

The Attached Form and the Origin of Radial Symmetry.

With almost no exception, students of the embryology and anatomy of the echinoderms see no other way, at present, to account for the peculiar asymmetry of certain of the organs and

the perfect radial symmetry of other structures, which are characteristic of the adult condition and which arise, in every known case, by the remodelling of the structures of a larva which is bilateral in its entire organization, than by assuming that the group has been derived from a bilateral pelagic organism, similar to the one described above, which at a very remote period in its existence exchanged its free swimming life for one on the bottom during which it became fixed.

Briefly stated, the steps by which the present organization of an echinoderm are generally accounted for, and for which there is more or less evidence, are as follows: Pelagic life was given up for one on the bottom because of less competition and a greater food supply in the latter place. The preoral lobe became gradually modified into an organ for fixation. The mouth, at first directed downward as was its position in pelagic life, gradually moved to the left until it took up a position in which it was directed upward. This, in a fixed animal, feeding upon microscopic organisms, is its most favorable position as is shown by its position in animals which exist at present under these conditions. During the migration of the mouth and œsophagus, those organs of the left side which would obstruct such a movement (left middle and posterior body cavities) were carried along and each became drawn out into the shape of a horseshoe and greatly hypertrophied. In the final position of each, the opening of the horseshoe was directed anteriorly. The middle and posterior body cavities of the right side also became changed in position and correspondingly reduced in size. The left middle body cavity retained its connection with the exterior through the greatly reduced anterior left body cavity and its duct, but the duct of the right side disappeared. During this period when food was plentiful and easily accessible and when no energy was used in locomotion, a rapid increase in the size of the creature took place and radial symmetry was developed. There is such a diversity of opinion, however, as to the details of this process that I will attempt to give but one; that which has been suggested by my own observations.

The ciliated rings were useful to the free-swimming animal not only as organs of locomotion but were used in feeding as well,

and during the period when it was fixed on the bottom certain of the rings continued to function as food gatherers. The two rings which encircled the preoral lobe, being purely locomotor in function, were lost, but the other three took up a more definite relation to the mouth and formed six paths for conducting food to it (see Fig. 11, *b*). The retention of the ciliated rings among directly developing larvæ and the return to a condition with ciliated rings among larvæ which possess a more complicated ciliated apparatus during their free-swimming life may, as I have stated elsewhere, be explained on this ground. The entire number of rings is not in every ease retained or reproduced because two, Nos. 1 and 2, belonged entirely to the locomotor apparatus (preoral lobe) and except in the holothurians and crinoids (for reasons suggested later) are no longer needed. Only those rings are retained which in the ancestral line had a function in feeding, and which are needed for the same purpose during the metamorphosis of the larvæ themselves until the developing tube feet are ready to assume the function.

This ciliated feeding apparatus which had been brought over by the hypothetical fixed echinoderm from its free-swimming condition and which, in the new surroundings, had, at first, answered every need in this line, became gradually inadequate to furnish it, as it increased in size, with enough food. Those portions of the ciliated sensory epithelium of the mouth situated between the ends of the ciliated paths were then gradually developed into tentacles into each of which a diverticulum of the left middle body cavity, lying below, protruded (see Fig. 11, *b*). In the anterior space only, did no tentacle develope. This space contained the external opening of the left middle body cavity (madreporite), the left anterior body cavity (*Ampulla*) and possibly the reproductive organ. There was hence no space in which a sixth tentacle might have developed.

In this way the pentamerous structure of the hydrocœle may be accounted for and I assume, with others, that the hydrocœle formed the basis upon which the entire radial symmetry of echinoderms was built. The ciliated tentacles, simple at first, branched as they grew in length and assumed more and more the function of food collecting. As the animal increased in size the space

immediately surrounding it failed to yield a sufficient supply of food. The tentacles in reaching about over the bottom in search of more, detached the animal and a crawling habit was developed. As the tentacles grew in length and complexity a like development in the organs which nourish, enervate, support and protect them would naturally follow. The tentacles being five in number, we have in them a possible origin for the pentamerous symmetry which characterizes the nervous and skeletal systems and a considerable part of the cœlomic cavities of all echinoderms. At the time when fixed life was given up by the ancestor of those echinoderms which are at present free living, each of its radii probably contained a five-branched tentacle, since this is the number which is possessed by many echinoderms at the period when their metamorphosis is being completed. The period of fixation was long enough and the changes which took place in the organization of the animal at this time were so great that all trace of an *anterior* or a *posterior* part, as such, was lost and now, in its second period of free life, the direction of locomotion depends wholly upon external conditions.

During the period when the common ancestor of the group was fixed, differentiations into at least three different types took place. One line is now represented by holothurians, one by crinoids and another by asterids, echinoids and ophiuroids. Among crinoids alone the fixed condition has been retained. In this group the problem of enlarging its base of supplies was solved not by becoming free but by the elongation of the organ of attachment, and by the migration of the mouth and tentacles still further toward the opposite end. In the type which has given rise to holothurians, the mouth and tentacles migrated in just the opposite direction, *viz.*, into the organ of attachment and were thereby brought into relation with the bottom. The free-crawling habit was later acquired. The ancestor of the starfishes and sea-urchins made no permanent use of its organ of attachment and no further migration of the mouth took place but it was brought into direct relation to the bottom by the rotation of the body as a whole.

Zoölogical Laboratory of the Johns Hopkins University,
April, 1903.